Effect of Inflammatory Cytokines on DNA Methylation and Demethylation

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INTRODUCTION

Periodontitis is a common cause of tooth loss and contributes to other common conditions. It affects 46% of adult Americans, 8.9% of them severely[1]. Bacteria are essential for initiation, but host factors are crucial for development of chronic inflammation. An imbalance exists between inflammatory and anti-inflammatory cytokines, and increased levels of IL-1β, IL-6 and TNFα activate gingival fibroblasts to produce inflammatory mediators including prostaglandin E2 (PGE2) and matrix metalloproteinases (MMPs), which contribute to continuing inflammation and destruction of tissues supporting teeth[2-6].

Periodontitis causes a chronic, low-grade systemic inflammation that contributes to development of other conditions, including cardiovascular disease, pre-term low birth weight, diabetes and several types of cancer[7-14]. Evidence is accumulating that epigenetic changes in inflamed tissues alter gene expression patterns to contribute to persistence of inflammation and to the predisposition to cancer associated with chronic inflammatory conditions[10, 15].

Here we show that both inflammatory cytokine IL-1 and inflammatory mediator PGE2 inhibit expression of the de novo methyltransferase DNMT3a as well as Ten Eleven Translocation 1 (TET1) in human gingival fibroblasts derived from patients with periodontitis, and present evidence that the effects of IL-1 may be mediated at least partly through the COX-2/PGE2 pathway. The results so far are consistent with the idea that chronic exposure of fibroblasts to inflammatory signaling can result in changes in DNA methylation/demethylation. These changes could result in global and/or gene specific changes which over time might contribute to the persistence of inflammation.

METHODS

Cell culture - Human gingival tissue samples are obtained with informed consent from patients receiving treatment for periodontitis under a protocol approved by PCOM's IRB (protocol #H04-015). The gingival tissue is processed by enzymatic dispersion to produce primary cultures. Cells are maintained in Eagle's Minimal Essential Medium (EMEM) supplemented with 10% fetal bovine serum and antibiotic/antimycotic (penicillin, streptomycin, amphotericin; Gibco/BRL). Cells between passages 3 and 5 are used for experiments.

RNA Isolation and Analysis - -- Total RNA was isolated from cells untreated (Controls) or treated with IL-1 (10 ng/ml) or IL-6 (10 ng/ml), NS-398 (1 μM) of PGE2 (0.1-100 μM) for the indicated times. Two μg RNA was converted to cDNA using the High Capacity cDNA Archive Kit (Applied Biosystem) and Thermal Cycler Genius. Two μl of cDNA was used with Taqman Universal PCR Master Mix (Applied Biosystem), and probes for detection of DNMT1, DNMT3a, TET1 and Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH; Applied Biosystem). Real Time PCR was performed using the Applied Biosystem Prism 7000 Real Time PCR system. Reactions were done in quadruplicate and results were calculated according to the ΔΔCT method, and normalized to levels of GAPDH. Statistical significance was determined by One-way ANOVA and post-hoc Bonferroni.

RESULTS & DISCUSSION

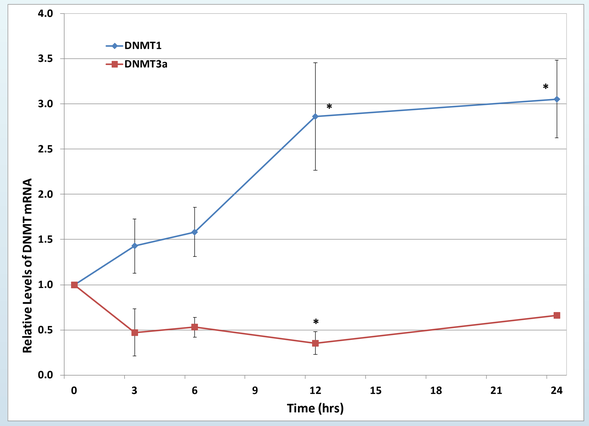

Figure 1. IL-1 induces DNMT1 and inhibits DNMT3a mRNA expression in HGF -- Total RNA was isolated from HGF cells treated with IL-1 (10 ng/ml) for the indicated times. DNMT1 and DNMT3a mRNA levels were quantified by real-time PCR, normalized to levels of GAPDH mRNA, and expressed relative to levels in the untreated cells harvested at time 0. The graph represents data from 5 independent experiments using HGF cultures derived from samples from 5 different individuals, average +/- SEM. Statistical significance was determined using One Way ANOVA with post-hoc Bonferroni. * p<0.05.

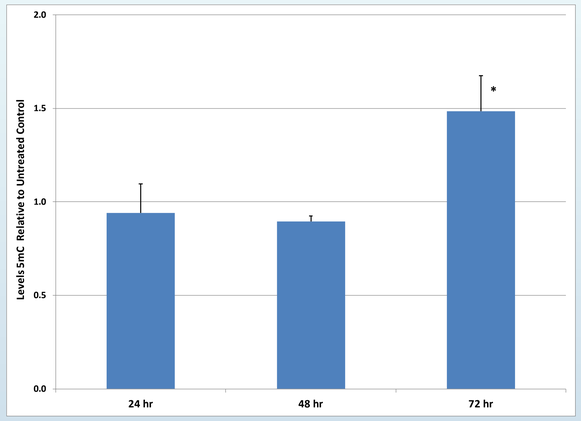

Figure 2. IL-1 increases global DNA methylation in HGF
HGF cells were left untreated or treated with IL-1 (10 ng/ml) for the indicated times. Levels of 5mC were measured using the MethylFlash Methylated DNA Quantification Kit (Epigentek), and expressed as treated relative to time 0 control.

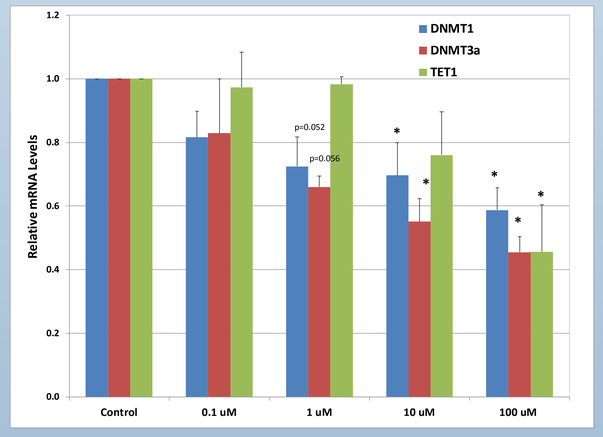

Figure 3. PGE2 causes dose-dependent inhibition of DNMT1, DNMT3a and TET1 mRNA in HGF – Total RNA was isolated from HGF cell cultures treated for 24 hours with the indicated doses of PGE2. DNMT1, DNMT3a and TET1 mRNA levels were quantified by real-time PCR, normalized to levels of GAPDH mRNA, and expressed relative to levels in the untreated control. The graph represents data from 4 independent experiments using HGF derived from 4 different individuals, average +/- SEM. Statistical significance was determined using One Way ANOVA with post-hoc Bonferroni. * p<0.05.

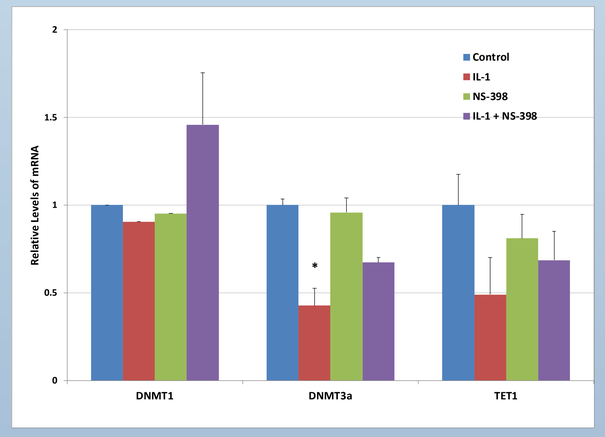

Figure 4. IL-1 inhibition of DNMT3a expression may be partially reversed by inhibition of COX-2 – Total RNA was isolated from HGF cell cultures treated for 24 hours withIL-1 (10 ng/ml), NS-398 (1 μM) or IL-1 + NS-398. DNMT1, DNMT3a and TET1 mRNA levels were quantified by real-time PCR, normalized to levels of GAPDH mRNA, and expressed relative to levels in the untreated control. The graph represents data from 4 independent experiments using HGF derived from 4 different individuals, average +/- SEM.

CONCLUSION

- These data provide evidence that both IL-1 and PGE2 affect expression of the de novo DNA methyltranserase DNMT3a and the demethylating enzyme TET1 in HGF.
- Treatment of HGF cultures with PGE2 caused a dose-dependent decrease in mRNA levels of DNMT1, DNMT3a and TET1 as compared to untreated controls.
- DNMT3a expression was inhibited by IL-1 regardless of when the control sample was isolated, but DNMT1 was induced by IL-1 only as compared to a time zero control. This suggests that expression of DNMT1 is affected by time in culture rather than by IL-1 per se.
- Global levels of DNA methylation increased slightly over 72 hour treatment with IL-1 as compared to a time zero control. This is consistent with the changes observed in DNMT1 expression over time, and most likely do not reflect changes brought about by IL-1.
- Although inhibition of COX-2 with NS-398 seemed to partially reverse the effects of IL-1 on expression of DNMT3a and TET1, this effect is not statistically significant. It is unclear at this time whether the effects of IL-1 might be mediated at least partly by increased PGE2 production.
- Even modest changes in expression of these enzymes, if sustained over time, might be expected to have significant effects on gene expression patterns.
- Further experiments are needed to determine:
  - whether changes in DNMT3a and TET1 mRNA levels in response to IL-1 and PGE2 are reflected in changes in levels of protein and/or enzymatic activity
  - whether any resulting gene specific changes in DNA methylation can be identified
  - molecular mechanisms involved

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